Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/GB04/005394

International filing date: 17 December 2004 (17.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: GB
Number: 0329635.7
Filing date: 23 December 2003 (23.12.2003)

Date of receipt at the International Bureau: 02 March 2005 (02.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

INVESTOR IN PEOPLE

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed Andrew Gersey

Dated 19 January 2005

Patents Form 1/77

Patents Act 1977
(Rule 16)

The Patent Office Designs Copyright Trade Marks

THE PATENT OFFICE
SN
23 DEC 2003
RECEIVED BY FAX

23DEC03 E861890-1 D01091
P01/7700 0.00-0329635.7 ACCOUNT CHA

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

1. Your reference — MBUS 1677 GB

23 DEC 2003

2. Patent application number *(The Patent Office will fill in this part)* — 0329635.7

3. Full name, address and postcode of the or of each applicant *(underline all surnames)* — JOHNSON MATTHEY PUBLIC LIMITED COMPANY
2-4 COCKSPUR STREET
TRAFALGAR SQUARE
LONDON SW1 5BQ

Patents ADP number *(if you know it)* — 536268007

If the applicant is a corporate body, give the country/state of its incorporation — GB

4. Title of the invention
PROCESS FOR PURIFYING (-)-Δ^9-TRANS-TETRAHYDROCANNABINOL
unable to insert chemical symbols

5. Name of your agent *(if you have one)* — IAN CARMICHAEL WISHART

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)* — JOHNSON MATTHEY TECHNOLOGY CENTRE
BLOUNT'S COURT
SONNING COMMON
READING RG4 9NH

Patents ADP number *(if you know it)* — 3991411001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))
— YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document		
	Continuation sheets of this form	0
	Description	7
	Claim(s)	2
	Abstract	1
	Drawing(s)	1
10. If you are also filing any of the following, state how many against each item.		
	Priority documents	
	Translations of priority documents	
	Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
	Request for preliminary examination and search *(Patents Form 9/77)*	ONE
	Request for substantive examination *(Patents Form 10/77)*	
	Any other documents *(please specify)*	
11.	I/We request the grant of a patent on the basis of this application. Signature [signature] IAN C. WISHART Date 23 Dec 03	
12. Name and daytime telephone number of person to contact in the United Kingdom	MRS F E STRANGE 0118 924 2125	

5N

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 08459 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

DUPLICATE

PROCESS FOR PURIFYING (-)-Δ^9-TRANS-TETRAHYDROCANNABINOL

The present invention relates to a process for purifying (-)-Δ^9-trans-tetrahydrocannabinol. The compound is separated from a mixture of cannabinoids using a chromatographic technique.

(-)-Δ^9-trans-tetrahydrocannabinol is the active ingredient in marijuana. It is used therapeutically as an inhalant or an oral drug for stimulation of appetite among AIDS and cancer chemotherapy patients. Tetrahydrocannabinols (THCs) can be isolated from marijuana (a mixture of leaves and flowering heads of the plant *Cannabis Sativa*). Alternatively, THCs can be obtained by synthetic routes, e.g. as described in WO 02/096899. Enantiomerically pure THCs are required for formulation into drug products, but the purification of THCs, whether produced by isolation or synthesis, is challenging. The present inventors have sought to provide a process for providing enantiomerically pure (-)-Δ^9-trans-tetrahydrocannabinol ((-)-Δ^9-THC).

Accordingly, the present invention provides a process for separating (-)-Δ^9-trans-tetrahydrocannabinol from a mixture of cannabinoids wherein the process comprises at least one chromatographic step wherein a mobile phase passes through a stationary phase, characterised in that the stationary phase comprises a derivatised polysaccharide and the mobile phase comprises carbon dioxide.

The process uses the combination of a derivatised polysaccharide stationary phase and a carbon dioxide-containing mobile phase and surprisingly provides complete removal of the cannabinoid components cannabidiol and (-)-Δ^8-trans-tetrahydrocannabinol from (-)-Δ^9-THC.

Carbon dioxide has previously been used as an extraction fluid or eluent in processes for separating (-)-Δ^9-THC. Veress (Journal of Chromatography A, 668 (1994), 285-291) has disclosed a method of extracting (-)-Δ^9-THC and cannabidiol from marijuana using a supercritical fluid extraction process wherein the extracting fluid is carbon dioxide and the trapping column is Hypersil ODS octadecylsilica stationary phase. Webster et al (US 6,403,126) discloses a method of preparing a

Cannabis extract wherein a supercritical fluid such as carbon dioxide may be used as an extractant. Cole ("Analysis of Cannabis by Supercritical Fluid Chromatography with Ultraviolet Detection", pages 145-148 in "Supercritical Fluid Methods and Protocols" ed. by Williams and Clifford) discloses an analysis of cannabis products using supercritical fluid chromatography wherein the mobile phase is carbon dioxide and methanol, and the stationary phase is a cyanopropyl silica column. However, the disclosed processes do not completely remove cannabidiol and (-)-Δ^8-THC from (-)-Δ^9-THC, as has been achieved in the present invention.

Levin et al (Journal of Chromatography A, 654 (1993), 53-64) have disclosed a chromatographic method for separating THCs using a stationary phase which was a derivatised polysaccharide immobilised on silica. A Daicel Chiralpak-AD column, which is based on amylose tris(3,5-dimethylcarbamate) supported on macroporous silica gel, was used. The mobile phase was *n*-hexane with ethanol or 2-propanol. In the present invention, a mobile phase comprising carbon dioxide is advantageously employed. The carbon dioxide can be recycled or can alternatively be released to the atmosphere without treatment. The carbon dioxide is easily removed from the product, and also helps keep the product isolated from air by having dissolved carbon dioxide in the product solution. The carbon dioxide does not create a static charge in reaction vessels, as can be the case with hexane.

Preferably the mobile phase in the present invention is a mixture of carbon dioxide and one or more modifiers. The modifier can be any liquid solvent such as an alcohol, ethyl acetate, acetonitrile or methylene chloride. The modifier should be compatible with the stationary phase, e.g. ethyl acetate and methylene chloride cannot be used with a Chiralpak AD column as they will destroy the column. The modifier is suitably a C_1-C_5 alcohol, most preferably ethanol. A carbon dioxide and ethanol mobile phase has been found to be particularly advantageous. When (-)-Δ^9-THC is prepared according to the synthetic route outlined in WO 02/096899, one of the impurities is "DPA-iso" (see figure 1 for the chemical structure). When the mobile phase is carbon dioxide/ ethanol, the DPA-iso elutes before the (-)-Δ^9-THC. A minor impurity eluting in front of a major component usually focuses due to the effects of displacement chromatography, so it was possible to remove all of the DPA-iso. Using an alternative heptane/ethanol mobile phase, the DPA-iso eluted after the (-)-Δ^9-THC.

It is considerably more difficult to resolve minor components eluting in the tail of a major component, so the carbon dioxide/ethanol mobile phase provides a significantly improved process compared to heptane/ethanol.

Carbon dioxide is easily removed, so the (-)-Δ^9-THC product can be provided as a solution with the modifier as a solvent. It may therefore be desirable to choose a modifier in which the (-)-Δ^9-THC is stable.

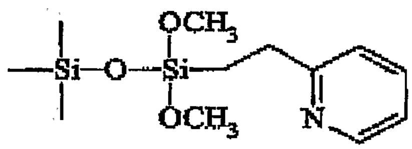
The ratio of carbon dioxide to modifier, as a weight (g) to volume (cm^3) is suitably in the range 100:1 to 50:50, preferably in the range 95:5 to 75:25, most preferably in the range 85:15 and 75:25. The ratio of carbon dioxide to modifier can be varied during the chromatographic process.

The stationary phase comprises a derivatised polysaccharide and is a solid chiral stationary phase. The derivatised polysaccharide is suitably immobilised on a substrate such as silica gel, zirconium, alumina, ceramics or other silicas, and is preferably immobilised on silica gel. Examples of derivatised polysaccharides include amylosic, cellulosic, chitosan, xylan, curdlan, dextran and inulan classes of polysaccharides. The amylosic polysaccharides are preferred. A particularly preferred stationary phase is amylose tris(3,5-dimethylphenylcarbamate) supported on macroporous silica gel, which is available as Chiralpak AD, manufactured by Daicel Chemical Co.

The stationary phase is preferably an encapsulated derivatised polysaccharide; the polysaccharide groups are not bonded to a substrate. It is thought that the encapsulated stationary phase may prevent decomposition of the (-)-Δ^9-THC to (-)-Δ^8-THC.

In a preferred embodiment of the invention the process comprises a further chromatographic step wherein a mobile phase passes through a stationary phase, wherein the stationary phase is an achiral stationary phase and is suitably selected from silica gel and derivatised silica gels, wherein the silica is derivatised with aminopropylsiloxane, diol-substituted propylsiloxane or 2-ethylpyridine siloxane groups. The 2-ethylpyridine siloxane immobilised on a silica support (shown below)

is a preferred achiral stationary phase because the (-)-Δ^9-THC does not degrade to form (-)-Δ^8-THC, as was observed with some achiral stationary phases.



The further chromatographic step ensures removal of the impurity (-)-Δ^9-abn-THC (see figure 1 for the chemical structure) from a mixture of cannabinoids.

In the further chromatographic step, the mobile phase suitably comprises carbon dioxide and is preferably a mixture of carbon dioxide and one or more modifiers. The modifier can be any liquid solvent but is suitably a C_1-C_5 alcohol, most preferably ethanol. The ratio of carbon dioxide to modifier, as a weight (g) to volume (cm^3) is suitably in the range 100:1 to 50:50, preferably in the range 95:5 to 75:25, most preferably in the range 95:5 and 90:10. The ratio of carbon dioxide to modifier can be varied during the chromatographic process.

Suitably a first chromatographic step uses the achiral stationary phase, preferably 2-ethylpyridine siloxane immobilised on a silica support and a second chromatographic step uses the derivatised polysaccharide stationary phase, and most preferably amylose tris(3,5-dimethylphenylcarbamate) supported on macroporous silica gel. It is preferred to use an amylosic stationary phase after a 2-ethylpyridine siloxane phase because it has been found that the amylosic phase can be destroyed by solvent impurities that might be present in the crude cannabinoid feed, whereas the 2-ethylpyridine siloxane is more robust. However, pure (-)-Δ^9-THC can also be achieved by reversing the two steps, i.e. using the 2-ethylpyridine siloxane phase after the amylosic phase.

Suitable chromatographic apparatus is well known to the skilled person. It is preferred to use apparatus that is suitable for Supercritical Fluid chromatography such as the Novasep Supersep 10 SFC or the Novasep Supersep 100 SFC. The crude feed containing the mixture of cannabinoids is periodically injected into the apparatus wherein the mobile phase flows through the stationary phase which is located in a

column. After detection at the column outlet, the purified fractions of the feed are directed to different traps. The carbon dioxide is removed from the purified fractions and is preferably recycled. Detection at the column outlet can be conducted by measuring UV absorption at an appropriate wavelength.

The column diameter is suitably from 0.5cm to 35cm and the column length is suitably from 5cm to 50cm. The particle size of the stationary phase is typically from 5 to 50μm.

The process is suitably carried out at temperatures from 5 to 45°C and at elevated pressures, e.g. from 80bar to 300bar. Typical flow rates depend upon the diameter of the column, and may vary from, e.g. 10g to 4kg/min.

The following examples are illustrative but not limiting of the invention.

Crude feed

(-)-Δ^9-trans-tetrahydrocannabinol was prepared as described in WO 02//096899. The crude reaction product included a variety of cannabinoid impurities, which are shown with (-)-Δ^9-trans-THC in figure 1. The crude reaction product was dissolved in ethanol to provide the crude feed.

Chromatographic apparatus

A Novasep Supersep 10 SFC was used in both chromatographic steps. Two stationary phases were used: a 2-ethylpyridine siloxane stationary phase, manufactured by Princeton Chromatography Inc, with a particle diameter of 10μm, and a Chiralpak AD stationary phase (amylose tris(3,5-dimethylphenylcarbamate) supported on macroporous silica gel), manufactured by Daicel Chemical Co., with a particle diameter of 20μm. The chromatographic steps were carried out at 25°C and at a pressure of 100bar.

Example 1: Two step purification using a 2-ethylpyridine siloxane column and a Chiralpak AD column

Step 1: Chromatographic separation using a 2-ethylpyridine siloxane column

The crude feed was filtered through 0.2 micrometer filter (Whatman PTFE w/GMF) and injected onto a chromatography column (length 25cm, inner diameter 2.1cm) containing 2-ethylpyridine siloxane stationary phase at a column flow rate of 40g/min using a mobile phase of 92% carbon dioxide and 8% ethanol. Column injections on the column were 0.85ml of crude feed injected for 5 seconds. After 128 column injections over 48 hours, an ethanolic solution was recovered with a (-)-Δ^9-trans-THC concentration of about 25g/l. After removal of ethanol by rotary evaporation at 30°C under vacuum, about 22.5g of semi-purified (-)-Δ^9-trans-THC was recovered exhibiting greater than 95% purity.

Step 2: Chromatographic separation using a Chiralpak AD column

The semi-purified (-)-Δ^9-trans-THC was re-dissolved in absolute ethanol to a concentration of about 300g/l to produce a feed for injection onto Chiralpak AD column (length 25cm, inner diameter 2.1cm) at a column flow rate of 40g/min using a mobile phase of 80% carbon dioxide and 20% ethanol. After 60 column injections of 0.85ml (over 5 seconds) of the semi-purified feed, an ethanolic solution was recovered (about 2.2 liters) and stored in a freezer for solvent evaporation at a later date. The estimated recovery of purified (-)-Δ^9-trans-THC (>99.5% purity) was ~15 grams.

Example 2: Two step purification using a Chiralpak AD column and a 2-ethylpyridine siloxane column

Step 1: Chromatographic separation using a Chiralpak AD column

The crude feed was filtered through 0.2 micrometer filter (Whatman PTFE w/GMF) and injected onto a chromatography column (length 25cm, inner diameter 2.1cm) containing Chiralpak AD stationary phase at a column flow rate of 40g/min using a mobile phase of 80% carbon dioxide and 20% ethanol. After 35 column injections of 0.85ml, 6g of semi-purified (-)-Δ^9-trans-THC (97.4% purity) were

collected. The majority of the remaining impurity was found to be (-)-Δ^9-abn-THC (2.5% AUC).

Step 2: Chromatographic separation using a 2-ethylpyridine siloxane column

The semi-purified (-)-Δ^9-trans-THC was injected onto Chiralpak AD column (length 25cm, inner diameter 1cm) at a column flow rate of 20g/min using a mobile phase of 92% carbon dioxide and 8% ethanol. After 3 second column injections at 5ml/min of the semi-purified feed, (-)-Δ^9-abn-THC impurity was reduced to less than 0.05% AUC.

Comparative Example 1: One step purification using a Chiralpak AD column and a hexane/ethanol mobile phase

The crude feed was filtered through 0.2 micrometer filter (Whatman PTFE w/GMF) and injected onto a chromatography column (length 25cm, inner diameter 1cm) containing Chiralpak AD stationary phase at a column flow rate of 4.8ml/min using a mobile phase of 95% hexane and 5% ethanol. Preparative purification of (-)-Δ^9-trans-THC from the DPA-iso impurity was not achieved.

Claims

1. A process for separating (-)-Δ^9-trans-tetrahydrocannabinol from a mixture of cannabinoids wherein the process comprises at least one chromatographic step wherein a mobile phase passes through a stationary phase, characterised in that the stationary phase comprises a derivatised polysaccharide and the mobile phase comprises carbon dioxide.

2. A process according to claim 1, wherein the mobile phase is a mixture of carbon dioxide and one or more modifiers.

3. A process according to claim 2, wherein the mobile phase is a mixture of carbon dioxide and ethanol.

4. A process according to claim 2 or claim 3, wherein the ratio of carbon dioxide to liquid modifier is in the range 95:5 to 75:25.

5. A process according to any preceding claim, wherein the derivatised polysaccharide is immobilised on a substrate chosen from silica gel, zirconium, alumina, ceramics and other silicas.

6. A process according to any preceding claim, wherein the stationary phase comprises an amylosic polysaccharide.

7. A process according to claim 6, wherein the stationary phase is amylose tris(3,5-dimethylphenylcarbamate) supported on macroporous silica gel.

8. A process according to any preceding claim, wherein the process comprises a further chromatographic step wherein a mobile phase passes through a stationary phase, wherein the stationary phase is an achiral stationary phase.

9. A process according to claim 8, wherein the achiral stationary phase is 2-ethylpyridine siloxane immobilised on a silica support.

10. A process according to claim 8 or claim 9, wherein a first chromatographic step uses the achiral stationary phase and a second chromatographic step uses the stationary phase comprising a derivatised polysaccharide.

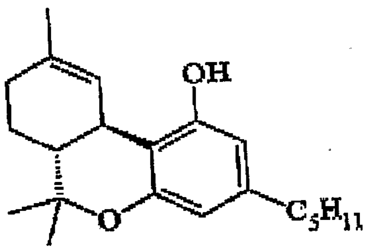
11. A process according to claim 8 or claim 9, wherein a first chromatographic step uses the stationary phase comprising a derivatised polysaccharide and a second chromatographic step uses the achiral stationary phase.

PROCESS FOR PURIFYING (-)-Δ^9-TRANS-TETRAHYDROCANNABINOL

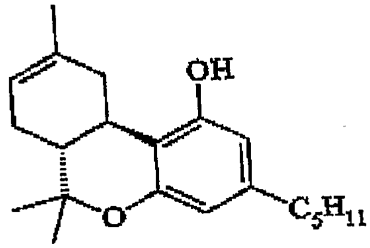
Abstract of the Invention

A process for separating (-)-Δ^9-trans-tetrahydrocannabinol from a mixture of cannabinoids is disclosed. The process comprises at least one chromatographic step wherein a mobile phase passes through a stationary phase. The stationary phase comprises a derivatised polysaccharide and the mobile phase comprises carbon dioxide.

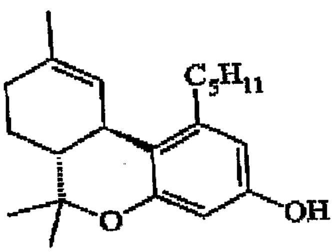
Figure 1



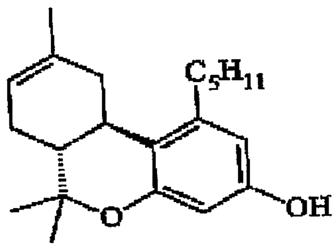
(-)-Δ^9-trans-THC



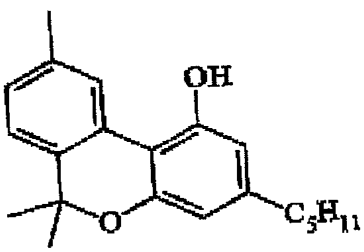
(-)-Δ^8-trans-THC



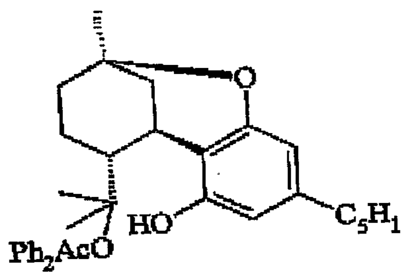
(-)-Δ^9-abn-THC



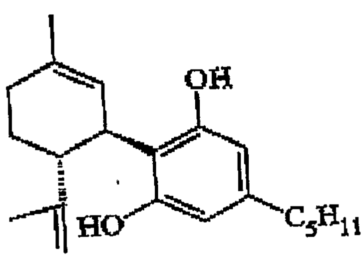
(-)-Δ^8-abn-THC



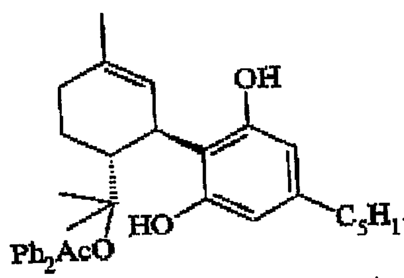
Cannabinol



"DPA-iso"



Cannabidiol



"DPA-triol"